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Investigation of the interaction of polyene antibiotics with cholesterol

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One of the compounds affected by membranes is polyene antibiotics. Amphotericin B, nystatin, mycoheptin, and levorin are mainly classified in the polyene class and distinguished by high biological activity. The high sensitivity of polyene antibiotics to membranes is due to the cholesterol they contain. The main feature of polyene antibiotics is the formation of structural ion channels by combining with cholesterol in the membranes. The interaction of polyene antibiotics with cholesterol has been demonstrated by obtaining ultraviolet (UV) spectra. Polyenes have three primary absorption spectra and range from 370 nm to 430 nm. Amphotericin B and levorin complex with cholesterol reduce the maximum amplitude of UV absorption spectra. The results show that cholesterol molecules combine with the double-bond chain systems of amphotericin B and levorin to reduce the maximum amplitude of UV absorption spectra. UV spectrum of dimethyl-sulfoxide molecules has been obtained. Its absorption spectrum ranges from 240 nm to 250 nm. The absorption spectrum of dimethyl sulfoxide molecules at these waves is related to the presence of the disulfide S=O group.

Keywords: *Levorin, amphotericin B, cholesterol, ultraviolet spectrum, cholesterol-polyene complex*

INTRODUCTION

One of the most significant problems of modern biophysics is to study at the molecular level of selective conductivity for ions and organic compounds in cell membranes. It knowns that the transport of ions and organic compounds through membranes is carrying out through channels of molecular size. Polyene class compounds are representing by amphotericin B, nystatin, mycoheptin, candidiasis, and levorin molecules. Among the antibiotics studied, amphotericin B and levorin are distinguishing by great membrane activity (Cavassin et al., 2021). The use of these antibiotics in research is not accidental. The main feature of polyene antibiotics is to make structural ion channels of molecular size in the membranes (Kamiński, 2014). Cellular channels have a high selective conductivity for ions, substrates, nucleic acids in molecular size (Samedova et al., 2018). The most significant characteristic of PAs is that they are very responsive to cholesterol molecules present in membranes (Kamiński, 2014; Srinivasarao et al., 2018). The primary solvent for PA is dimethyl sulfoxide (DMSO). It is using in many fields of molecular biology and biochemistry. Studies have shown for the first time that antibiotics are very soluble in water in a complex with DMSO and have high biological activity. The essential purpose of the article is to show the formation of the complex between PA and cholesterol in experimental studies using the method of UV spectroscopy.

MATERIALS AND METHODS

Bilayer lipid membranes are using to study the effect of PA on membranes at the molecular level. Bimolecular lipid membranes (BLM) are the essential representatives of living membranes. Membranes are making of phospholipids and present in the brain tissues of large and small horned animals. The formation of lipid membranes in the hollow

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part of a glass made of Teflon material reflects on paper (Samedova et al., 2018). The ultraviolet spectrophotometer was used in the study to determine the absorption spectrum of PA. Amphotericin B and levorin UV spectra were determined using a T92 + UV / VIS spectrometer.

RESULTS AND DISCUSSION

It shows that the interaction of polyene antibiotics with cholesterol and other sterols (ergosterol, 7-dehydrocholesterol) in lipids and cell membranes results in the formation of structural ion channels with different permeability (Fei et al., 2012). Sterins are one of the main structural components of living cell membranes. UV spectroscopy is using to determine the biological activity of antibiotics (Vyazmin et al., 2011). The B spectra of levorin and amphotericin differ in the three main absorption spectra. The absorption spectra of antibiotics range from 370 nm to 430 nm. UV absorption spectrum reflects the characteristic spectra of polyenes belonging to this class. UV spectroscopy based on the irradiation of a substance with monochromatic UV radiation, and the absorption spectra vary with wavelength. It is basing on the identification of individual substances, as well as their quantitative measurement. UV radiation has a biological effect on living organisms. UV radiation penetrates the tissues to a depth of 0.5-1.0 mm, activating biochemical processes. Many morpho-physiological and biochemical parameters of plant cells change under the influence of UV radiation. These changes depend on the stage of tissue development, its genotype, and radiation conditions (radiation duration and spectral composition) (Vyazmin et al., 2011). The main feature of PAs is that they create an absorption spectrum that differs by three maximums in UV waves. Levorin macrolactone ring has seven double bonds and incorporates a polyene chromophore in the UV spectrum of levorin - 358 nm-360 nm, 378 nm-380 nm, and 400 nm-403 nm. The UV spectrum at these wavelengths is typical of heptaen antifungal antibiotics (Cavassin et al., 2021). Levorin is composed of the components levorin A and levorin B (Szczeblewski et al. 2017). Levorin A and B as aromatic heptaen differ from each other in elemental composition and UV spectra (Szczeblewski et al., 2017). Some physicochemical properties of levorin components A and B are given in Table 1. In addition, Table 1 presents the UV spectra of levorin A and levorin B. In comparison, the difference between levorin A and levorin B is 5-6 nm.

Table 1. Physicochemical properties of levorin A and B

General formula	Levorin A $C_{59}H_{93}O_{22}N_2$	Levorin B $C_{52}H_{98}O_{23}N_2$
UV absorption spectrum		
λ, nm	340 358 378 400	450 790 900 800
E	342 363 382 406	375 620 980 950
The composition of the elements		
C	60,43	59,72
H	7,89	7,87
N	2,38	2,24
Neutralization coefficient	1180	1238
Distribution factor	0,8	7,6
Nitrogenous part of the molecule	p-aminoacetophenone, mycosamine	p- aminoacetophenone, mycosamine

Figure 1 shows the UV absorption spectra of levorin A and levorin B in DMSO. Figure 2 shows the UV absorption spectra of levorin and candidiasis in methanol.

As shown in Fig. 1 and Fig. 2, the UV absorption spectrum of levorin depends on the solvents of the PA molecules. In the DMSO, the UV absorption spectrum of levorin varies in the wavelength range of 325–425 nm (Fig/ 1), while in the methanol, the UV absorption spectrum of levorin varies in the wavelength range of 360–400 nm (Fig. 2). The UV absorption spectra of levorin and candidiasis in methanol are the same. The results show that this is due to the differences between the chromophore in polyenes and their macrolactone ring. Thus, unlike candidiasis, the p-aminoacetophenone group in levorin is located in the hydrophobic part of the molecule.

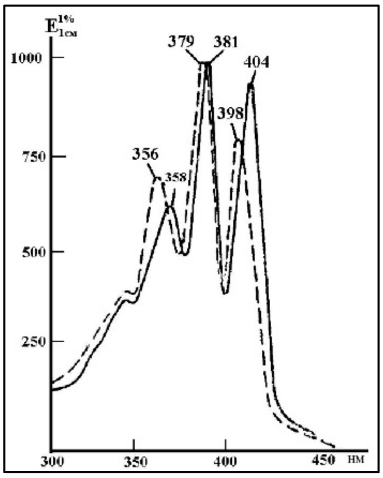


Fig. 1. UV absorption spectra of levorin A and levorin B in dimethyl sulfoxide.

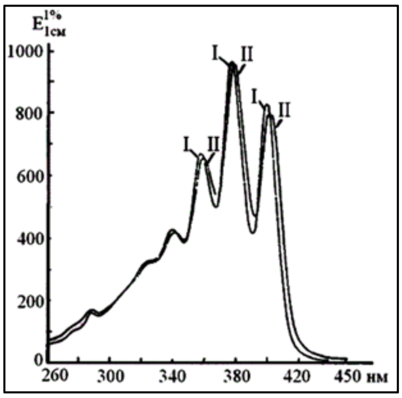


Fig. 2. UV absorption spectra of levorin and candidiasis in methanol: I - levorin; II – candidiasis.

Figure 3 shows the UV absorption spectrum of levorin and amphotericin B at different concentrations.

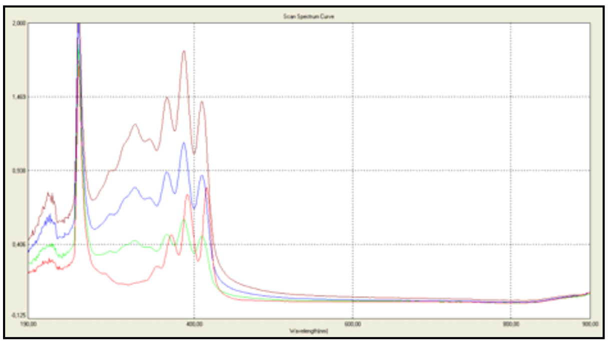


Fig. 3. UV absorption spectra of levorin were obtained in DMSO at different concentrations.

In Figure 3 (red curve), the UV absorption spectrum obtained at the final concentration of levorin 10^{-5} M by adding 0.3 ml and 3 ml of DMSO from the essential solution of levorin at a concentration of 1 mg/ml.

The blue curve was obtained by adding 0.2 ml and 3 ml of DMSO from the essential solution of levorin at a concentration of 1 mg/ml to obtain a UV absorption spectrum at a concentration of levorin $7 \cdot 10^{-6}$ M. The UV absorption spectra was obtained by adding 0.1 ml and 3 ml of DMSO to the green solution of levorin at a concentration of 1 mg/ml and levorin at a concentration of $3 \cdot 10^{-6}$ M. A bright red line was obtained by adding 0.03 ml and 3 ml of DMSO from the essential solution of levorin at a concentration of 1 mg/ml to obtain a UV absorption spectrum at a concentration of levorin $1 \cdot 10^{-6}$ M. As shown in Figure 3, the concentration of levorin increases with the amplitude of the UV-maximum absorption spectrum. Levorin absorption spectra range from 370 nm to 430 nm. UV absorption spectra reflect the characteristic spectra of polyenes belonging to this class. UV spectra of PAs obtained at different concentrations are one of the methods of reflecting their biological activity. Although polyenes are well soluble in DMSO, they are in the form of thin colloidal dispersions in water, whereas molecules in ethyl, methyl alcohol solutions, and water find their presence in disperse form (Pinisetty et al., 2012).

Figure 4 shows the UV absorption spectra of levorin derivatives, N-diacetyl-levorin sodium salt, levorin succinyl sodium salt, and levorin soluble in water and methanol.

Figure 4 shows that levorin derivatives are distinguishing by their biological activity. The highest biological activity shows by the sodium salt of levorin in methanol, spectrum 3, but the biological activity in water is low. The amphoteric nature of levorin is due to the presence of one carboxyl and two amine groups in its molecule. It allows its derivatives to remain in both alkaline and acidic environments. Figure 5 shows the UV absorption spectrum of DMSO.

One tub contains 3 ml of ethanol and the other tub contains 3 ml of DMSO. Temperature 24ºC. As can be seen in Figure 5, the absorption spectrum of the DMSO is reflected in the near-UV absorption waves. The UV spectrum of the DMSO is selected with a maximum absorption between 240 nm-250

nm. The gain spectrum of dimethyl sulfoxide molecules in these waves is due to the presence of the disulfide S=O group. Study of the complex formed by cholesterol with polyenes by UV spectroscopy.

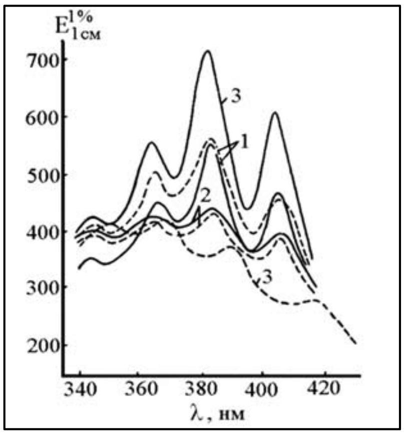


Fig. 4. UV absorption spectra of levorin derivatives soluble in water and methanol. - - in water; - methanol; 1 – N-diacetyl sodium salt of levorin; 2 – levorin succinyl sodium salt; 3 – sodium salt of levorin.

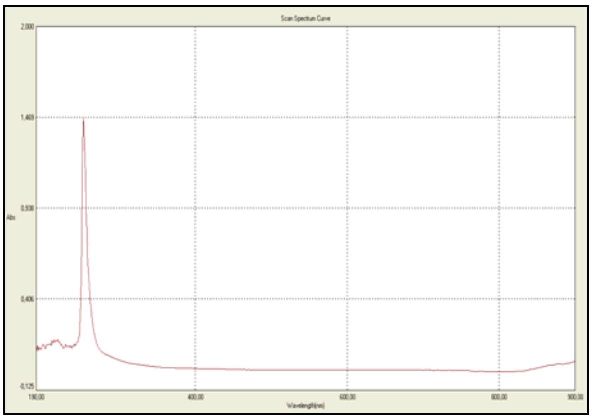


Fig. 5. UV differential absorption spectrum of DMSO.

PA is characterized by a maximum of three absorptions due to the presence of a double-bonded molecule in the chromophore part in water and organic solutions. Changes in the UV absorption spectrum observed during the interaction of PA with cholesterol. An increase in the number of double bonds in a polyene molecule causes a change in the UV absorption spectrum. The addition of cholesterol or other sterols to an antibiotic solution leads to a decrease in the UV absorption spectrum, resulting in the formation of a complex between cholesterol and PA. The presence of sterols does not change the UV absorption wavelength of polyenes and only changes the maximum UV absorption.

It has been shown that the addition of cholesterol to the aqueous solution of the Philippines changes the UV spectrum, but this member of the spectrum does not change in the solvents. No change in the UV spectrum occurs when cholesterol is added to an organic solvent. According to the effectiveness of interaction with cholesterol, PA is in the following order: Philippine > amphotericin B > etruscomycin > pimarisin > nystatin. The structure of sterols often determines their interaction with polyenes. Thus, sterols containing the 3β-OH group are more sensitive to polyenes than sterols containing 3α-OH or 3-keto groups. For polyenes to interact with sterols, the antibiotic C17 must form a hydrogen bond with the 3β-OH group of the sterins molecule. Table 2 shows the maximum UV absorption depending on the number of double bonds of the polyene. Figure 6 shows the UV absorption spectra of amphotericin interacting with B cholesterol.

Table 2. Maximum UV absorption of polyenes depending on the number of double bonds.

	Number of double bond	**The three maximum absorption in the UV, nm**	**Color**
Trien	3	-	-
Tetrans	4	291, 304, 318	Light yellow
Pentans	5	317, 331, 350	Yellow
Hexans	6	340, 358, 380	Yellowish-orange
Heptans	7	361, 382, 405	Orange

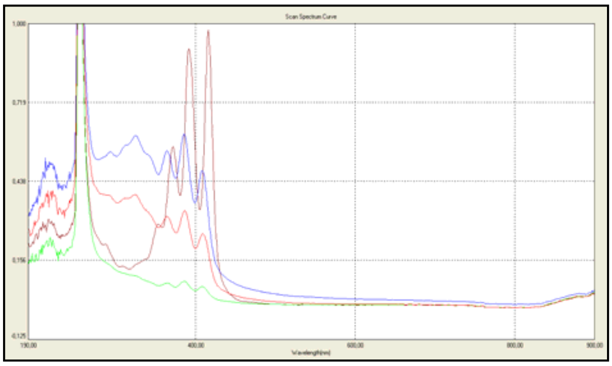


Fig. 6. UV absorption spectra of the interaction of amphotericin with B cholesterol.

In Fig. 6, a dark red line - UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B dissolved in 1 mg/ml DMSO to 3 ml of DMSO in the first tub. 3 ml of ethanol solution was added to the second tub. The blue line in Fig. 6 shows that the UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B dissolved in 1 mg/ml DMSO to the first tub and 0.5 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub. In Figure 6, a bright red line - UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B dissolved in 1 mg/ml DMSO to the first tub and 1 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub. In Figure 6, the green line - UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B soluble in 1 mg/ml DMSO to the first tub and 2 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub.

The results in Figure 6 show that amphotericin interacts with B cholesterol to reduce the concentration of amphotericin B, which is reflected in the UV absorption spectra.It is known that DMSO molecules facilitate the delivery of drugs from biological membranes to the cell. However, the effects of DMSO on membranes have not yet been fully studied (Lee et al., 2016).

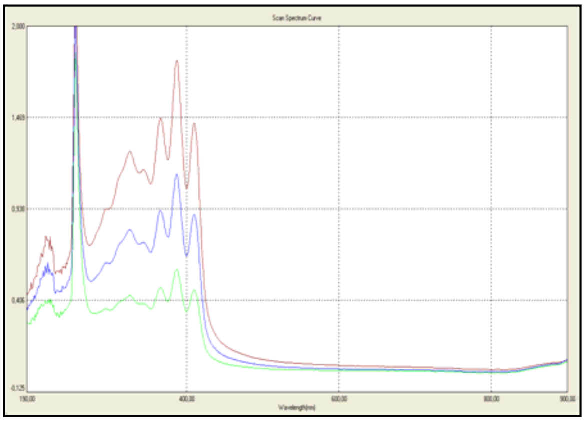


Fig. 7. UV absorption spectra obtained from the interaction of levorin with cholesterol.

It recently has been shown by molecular-dynamic modeling that DMSO creates water pores in biological membranes (Pinisetty et al., 2012). The effect of DMSO on the diffusion of Ca2 + ions from cell membranes has been studied(Jacl et al., 2014). The increase in DMSO-induced Ca2 + permeability did not alter the increase in Ca2 + permeability due to the effects of K-channel blockers and K-Na-ATF-aza. This means that water pores form in the cell membranes induced by DMSO, and Ca2 + ions are transferred to the cells through these pores. In addition, the permeability of Ca2 + ions increases significantly due to the high concentration of DMSO, which indicates the selectivity of water pores induced by DMSO. Thus, these studies suggest that DMSO can induce water pores in cell membranes and, in turn, facilitate the transport of biologically active substances to cells.

Figure 7 shows the UV absorption spectra of levorin were obtained from the interaction with cholesterol.

In Fig. 7 a dark red line - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO added to 3 ml of DMSO in the first tub. 3 ml of ethanol was added to the second tub.

The blue line in Fig. 7 - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO and 1 mg of cholesterol in 3 ml of DMSO added to the first tub. 3 ml of ethanol added to the second tub.

The green line in Fig. 7 - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO and 3 mg of cholesterol in 2 ml of DMSO added to the first tub. 3 ml of ethanol added to the second tub.

As shown in Fig. 6 and Fig. 7, amphotericin B and levorin form a complex with cholesterol, reducing the maximum amplitude of UV absorption spectra. Increased cholesterol further lowers the maximum of the UV absorption spectra. The results show that cholesterol molecules combine with the double communication systems of amphotericin B and levorin to gradually lower the maximum absorption spectra of UV. These studies confirm the complex formation of amphotericin B and levorin with cholesterol and the molecular model of the channel (Kaminski, 2014; Cavassin et al., 2021).

RESULTS

The ultraviolet spectra of amphotericin B and levorin show that amphotericin B differs from 370 nm to 420 nm and levorin from 368 nm to 410 nm with three essential absorption spectra, which are due to the presence of a chromophore chain in the molecule. Amphotericin B and levorin complex

with cholesterol reduce the maximum amplitude of UV gain spectra. The results show that cholesterol molecules combine with the double communication systems of amphotericin B and levorin to reduce the maximum amplitude of UV gain spectra. The ultraviolet spectrum of dimethyl sulfoxide molecules was obtained. Its gain spectrum ranges from 240 nm to 250 nm. The gain spectrum of dimethyl sulfoxide molecules in these waves is due to the presence of the disulfide S=O group.

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Polien antibiotiklərinin xolesterinlə qarşılıqlı təsirinin tədqiqi

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Membranlara təsir göstərən birləşmələrdən biri polien antibiotikləridir. Polien sinfinə daxil olan və yüksək bioloji aktivliyi ilə seçilən əsasən amfoterisin B, nistatin, mikoheptin və levorindir. Poliyen antibiotiklərinin membranlara olan yüksək həssaslıgı onların tərkibindəki xolesterinlə baglıdır. Polien antibiotikləri əsas xüsusiyyəti membranlarda xolesterinlə birləşərək struktur-ion kanallarının yaradılmasıdır. Polien antibiotiklərin xolesterinlə qarşılıqlı təsiri ultrabənövşəyi (UB) spektrlərinin alınması ilə göstərilmişdir. Polienlər

üç əsas udlu spektri ilə fərqlənir və 370 nm – 430 nm çərçivəsində dəyişir. Amfoterisin B və levorin xolesterinlə kompleks yaradaraq UB udlu spektrlərinin maksimum amplitudasını aşağı salır. Alınan nəticələr onu göstərir ki, xolesterin molekulları amfoterisin B və levorinin qoşa rabitə sistemləri ilə birləşərək UB udma spektrlərinin maksimum amplitudasını azaldır. Dimetilsulfoksid molekullarının UB spektri alınmışdır. Onun udma spektri 240 nm – 250 nm dalğalarının uzunluqu arasındadır. Dimetilsulfoksid molekullarının göstərilən dalğalarda udma spektri disulfid S=O qrupunun mövcud olması ilə bağlıdır.

Açar sözlər: *Levorin, amfoterisin B, xolesterin, ultrabənövşəyi spektri, xolesterin-polien kompleksi.*

Исследование взаимодействия полиеновых антибиотиков с холестерином

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Одним из соединений, влияющих на мембраны, являются полиеновые антибиотики. В основном это амфотерицин В, нистатин, микогептин и леворин, которые относятся к классу полиенов и отличаются высокой биологической активностью. Высокая чувствительность полиеновых антибиотиков к мембранам обусловлена содержащимся в них холестерином. Главной особенностью полиеновых антибиотиков является создание структурно-ионных каналов в мембранах путем их соединения с холестерином. Взаимодействие полиеновых антибиотиков с холестерином показано путем изучения ультрафиолетовых (УФ) спектров. Полиены различаются по трем основным спектрам поглощения, которые варьируют в диапазоне 370 Нм – 430 Нм. Амфотерицин В и леворин снижает максимальную амплитуду УФ спектра, благодаря взаимодействию с холестерином. Уменьшение максимальной амплитуды спектров УФ поглощения есть результат взаимодействия холестерина с системой двойных связей амфотерицина В и леворина. Показано, что УФ-спектр молекул диметилсульфоксида находится в диапазоне длин волн 240 Нм – 250 Нм. Предполагается, что полученный спектр поглощения молекул диметилсульфоксида в указанных длинах волн обусловлен наличием в молекуле дисульфидной S=O группы.

Ключевые слова: *Леворин, амфотерицин В, холестерин, ультрафиолетовый спектр, холестерин-полиеновый комплекс*